

AD_____

Award Number: DAMD17-99-1-9148

TITLE: Breast Cancer Therapeutics, Environmental Estrogens, and the Estrogens Receptor (ER); Characterization of the Diverse Ligand Binding Properties of the ER

PRINCIPAL INVESTIGATOR: Deborah S. Wuttke, Ph.D.

CONTRACTING ORGANIZATION: University of Colorado
Boulder, Colorado 80309

REPORT DATE: July 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20020215 070

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)**2. REPORT DATE**

July 2001

3. REPORT TYPE AND DATES COVERED

Annual (1 Jul 00 - 30 Jun 01)

4. TITLE AND SUBTITLE

Breast Cancer Therapeutics, Environmental Estrogens, and the Estrogens Receptor (ER); Characterization of the Diverse Ligand Binding Properties of the ER

5. FUNDING NUMBERS

DAMD17-99-1-9148

6. AUTHOR(S)

Deborah S. Wuttke, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Colorado
Boulder, Colorado 80309

E-Mail: Deborah.Wuttke@Colorado.EDU**8. PERFORMING ORGANIZATION
REPORT NUMBER****9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING
AGENCY REPORT NUMBER****11. SUPPLEMENTARY NOTES**

Report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE**13. ABSTRACT (Maximum 200 Words)**

The estrogen receptor (ER) is found in the nucleus of several tissues, including breast, bone, liver, the organs of the reproductive system, and the cardiovascular system. The ER binds several types of compounds, including compounds that are quite distinct from its natural ligand. Estrogens bind to and activate the ER, which leads to the stimulation of transcription of genes containing an estrogen responsive element (ERE). Antiestrogens and partial antiestrogens bind tightly to the ER but fail to activate transcription; these compounds are currently in widespread use for the treatment of breast cancer. In addition, a variety of compounds introduced into the environment by human activity have also been found to act as estrogen mimics and alter reproductive function and development. The goals of this project are to understand, on a molecular level, how the ER binds estrogens, anti-estrogens, and estrogen mimics present in the environment, how this binding triggers activity, and how mutations in the ER discovered in breast cancer patients affect ER activity. Towards this end, we have expressed and purified the ligand binding domain of the estrogen receptor, and studied complexes with estradiol, the natural ligand, and tamoxifen, a partial antiestrogen in use as a breast cancer therapeutic using high-resolution heteronuclear NMR spectroscopy.

14. SUBJECT TERMS

Breast Cancer, Estrogen Receptor, Nuclear Magnetic Resonance Spectroscopy, Estradiol, Tamoxifen

15. NUMBER OF PAGES

21

16. PRICE CODE**17. SECURITY CLASSIFICATION
OF REPORT**

Unclassified

**18. SECURITY CLASSIFICATION
OF THIS PAGE**

Unclassified

**19. SECURITY CLASSIFICATION
OF ABSTRACT**

Unclassified

20. LIMITATION OF ABSTRACT

Unlimited

**Annual Report for Breast Cancer Therapeutics, Environmental Estrogens,
and the Estrogen Receptor (ER): Characterization of the Diverse Ligand
Binding Properties of the ER**

Deborah S. Wuttke
Department of Chemistry and Biochemistry
University of Colorado, Boulder

TABLE OF CONTENTS

Front Cover	1
Standard Form (SF) 298	2
Table of Contents	3
Introduction	4
Body	5
Key Research Accomplishments	9
Reportable Outcomes	9
Conclusions	10
References	10
Appendices	10

INTRODUCTION

The estrogen receptor (ER) is found in the nucleus of several tissues, including breast, bone, liver, the organs of the reproductive system, and the cardiovascular system. The ER binds several types of compounds, including compounds that are quite distinct from its natural ligand. Estrogens bind to and activate the ER, which leads to the stimulation of transcription of genes containing an estrogen responsive element (ERE). Antiestrogens and partial antiestrogens bind tightly to the ER but fail to activate transcription; these compounds are currently in widespread use for the treatment of breast cancer. In addition, a variety of compounds introduced into the environment by human activity have also been found to act as estrogen mimics and alter reproductive function and development. The estrogenic behavior of these compounds has proven difficult to predict from their structures; many of these hormone mimics bear little structural resemblance to natural estrogens. The molecular level details of the conformational changes that allow the ER to tightly bind a diverse array of compounds and result in diverse patterns of gene activation are not understood. The goals of this project are to understand, on a molecular level, how the ER binds estrogens, anti-estrogens, and estrogen mimics present in the environment, how this binding triggers activity, and how mutations in the ER discovered in breast cancer patients affect ER activity. A complete structural understanding of how various ligands interact with the ER, and are able to elicit different responses, will assist in identifying compounds with therapeutic benefit for treating breast cancer. In addition, our studies will contribute to understanding the effects environmental estrogens have on breast cancer, and in identifying estrogenic activity in compounds before they are introduced into the environment.

BODY

The large numbers of compounds which bind the ER will require the use of an innovative and rapid approach. We are investigating ligand binding to the ER using multidimensional nuclear magnetic resonance spectroscopy (NMR) studies of complexes of these compounds with the ligand binding domain (LBD) of the ER. The ER-LBD is uniformly isotopically labeled with ^{13}C and ^{15}N and ligand binding will be followed by acquiring heteronuclear single quantum coherence (HSQC) spectra, which are exquisitely sensitive to molecular conformation. Also, we will study the structural effects of several point mutations isolated from breast cancer cell lines. We will investigate the ligand binding differences between $\text{ER}\alpha$ and $\text{ER}\beta$, the two natural variants of the ER. These differences may play an important role in the tissue specific activity of partial antiestrogens. Analysis of our NMR data will be significantly aided by the present availability of the crystal structures of the ER bound to both estrogens and antiestrogens. The use of NMR spectroscopy as an efficient tool for screening for compounds with estrogenic or antiestrogenic activity, or with activity specific to either $\text{ER}\alpha$ or $\text{ER}\beta$ will be explored.

Progress in the last year in the first 3 technical objectives has been achieved. This progress towards the tasks in the statement of work are as follows:

The tasks associated with technical objective 1 were mostly achieved within the first year of the grant, however, for a variety of reasons we have needed to develop more reliable and flexible protein expression and purification strategies. The LBD-ER was prepared by expression in BL21(DE3) *E. coli*

by induction with IPTG from a pET plasmid. Complete purification was achieved using an estradiol affinity column that afforded protein of high purity and activity in one step. In addition, the purified protein is highly stable to proteolysis, a problem that rendered protein purified by other strategies inactive within a few days of preparation. Please refer to figures in the Appendix for protein induction and purification gels. A complete buffer screen revealed optimal buffer conditions (20mM d-Tris, 100mM NaCl, 100mM Na₂SO₄, 1mM d-DTT, 0.02% sodium azide) that allowed us to prepare samples with protein concentrations of up to 500 μ M. Homonuclear NMR data obtained on these samples revealed features consistent with a protein of the anticipated molecular weight. The optimum temperature for data collection was determined to be 30°C. The anticipated molecular weight of the estradiol bound complex was confirmed by light scattering data and shown unambiguously to be a dimer. In the second year of the grant, we extensively re-evaluated the conditions for protein expression. We discovered that our previous protocol did not reliably produce protein, *i.e.*, in certain growths when protein expression was induced at high ODs no protein production was observed. This is presumably due to loss of ampicillin resistance at high bacterial cell concentration. This is not uncommon as the resistance to antibiotic is conferred by a secreted protein. Thus, induction at an OD of 1.0 at 600 nm was discovered to give completely reliable protein production. We have modified all of our protocols to accommodate this improvement.

As work proceeds on the project it will be important to have a ligand-free form of the ligand binding domain (LBD) of the estrogen receptor. This will allow us to examine structural differences between the free and bound forms and ultimately look at the amount of conformational change that occurs upon binding of the ligand. Our current purification strategy relied on an estradiol affinity column, and the protein necessarily is eluted with ligand. For weak binding ligands, this protocol will be very inefficient as most ligands are not soluble at the concentrations that are needed to compete the protein from the column. Thus, a stable, ligand-free preparation protocol is needed. Preliminary efforts at preparing the ligand free form of the LBD are modeled on a differential detergent solubilization method. After expression of the LBD using the standard technique, the pellet is first washed with a buffer containing the detergent octyl glucoside which preferentially solubilizes almost all the other cell proteins. After octyl glucoside extraction, the cell pellet is washed with a buffer containing the detergent Zwittergent 3-12, which then solubilizes the estrogen receptor LBD. Preliminary efforts at this procedure indicate that it does indeed purify significant amounts of the ER-LBD and it appears that after dialyzing out the detergent that the LBD refolds and regains the ability to bind estrogen. As time permits, this procedure for purifying ligand-free ER-LBD will be further optimized and structural studies on the ligand-free form will be pursued.

Growth of uniformly ¹⁵N labeled protein needed for Technical Objectives 2 and 3 progressed smoothly based on the expression and purification scheme developed for unlabeled protein. ¹⁵N-¹H HSQC NMR data were collected at 500 and 600 MHz. Significant improvement of data quality was observed at higher field strengths and with the addition of TROSY pulse sequences (see figures in Appendix). This project would not be feasible without the TROSY advances. Moreover, the enhancement of signal using TROSY increases as the field strength used for data collection increases. A tamoxifen-bound complex was prepared to compare tamoxifen to estradiol-bound forms of the protein. Significant chemical shift differences were noted between these two complexes, as anticipated, illustrating the validity of our strategy (see overlay of spectra in Appendix). These data were collected at the end of the first year of funding.

In order to interpret the chemical shifts in the context of available structures, resonance assignments need to be completed. Towards this end, uniformly ^{13}C , ^{15}N and ^2H labeled protein has been prepared. The ^{13}C and ^{15}N labels will allow assignment of the backbone resonances via their direct scalar couplings. Complete deuteration is employed to further reduce linewidths by eliminating the dominant mechanism of line broadening, relaxation via ^1H - ^1H dipolar couplings. To achieve this result, growth in D_2O has been optimized. A figure displaying SDS-PAGE analysis of our induction is shown in the Appendix. While yields are slightly reduced we clearly can obtain sufficient quantities for structural work. Our improved protein expression protocol has significantly enhanced our ability to prepare these labeled samples, as the D_2O growths are very expensive.

Our optimized protocol for preparation of fully deuterated, ^{13}C and ^{15}N labeled material follows: A single colony of transformed BL21(DE3)pLysS bacteria from a M9 minimal media plate was used to inoculate 1 mL M19 minimal media plus supplements containing $(^{15}\text{NH}_4)_2\text{SO}_4$, 3x glucose, 50 mg/mL carbenicillin (2x), 68 mg/mL chloramphenicol (2x), and made up in 70% D_2O . The culture was grown to saturation at 37 °C (over 17 hours). This culture was used to inoculate 10 mL of the same media, which was grown to an OD_{600} above 1.5, and then used to inoculate 100mL of media made up in 85% D_2O , with all the other same components. When the 85% D_2O culture reached an OD_{600} above 1.5 (approximately 10 hours), the shaker was cooled to 20 °C, and the 100mL culture was used to inoculate 1L of 99% D_2O media as above, except the $(^{15}\text{NH}_4)_2\text{SO}_4$ was increased to 1.5x. Increasing the glucose and $(^{15}\text{NH}_4)_2\text{SO}_4$ in the media increased yields. Adjusting the pH manually to 7.0 every 4-6 hours with 0.5 M NaOH made up in D_2O was tried during the growth. When the culture reached an OD_{600} of 1.0 (approximately 10 hours), ampicillin was added and the culture was induced with IPTG, as above, for 16-18 hours. Induction times ranging from 5 to 45 hours were tried, and this was found to produce optimal yields. The cells were then harvested as above. These growths produced 13.5 mg purified ER-LBD per liter.

Extensive efforts were aimed during the second year of funding at obtaining triple resonance data for resonance assignments. Pilot triple resonance experiments, including the HNCA and HNCOC, have been collected, and every possible parameter has been scrupulously optimized. However, the signal obtained on these experiments is not amenable to full resonance assignment. In fact, only about half of the anticipated resonance signals are observed. This is due to the large size of the ER-LBD, which is a symmetric dimer of molecular weight 56 kDa. Even with our advances using TROSY and deuteration, we have not been able to reduce the T_2 relaxation of the complex by a significant enough amount to prevent complete decay during the time of the pulse sequence. Unfortunately, the other triple-resonance experiments that are needed to make assignments, the HNCACO, HNCACB, CBCACONH etc, are even longer so the relaxation issues associated with large (*i.e.*, slowly tumbling systems) are even worse. Attempts to assign a partial data set such as this would be rather ill conceived, as it would be easy to make a mistake early in the assignment process that would lead to complete misassignment of the resonances. In fact, very few systems larger than 30 kDa have been assigned to date, highlighting the challenges associated with working with systems of this size. These studies indicate that further optimization, either through preparation of higher concentration samples or data collection at higher field, are necessary. In addition, we have begun to pursue other strategies for investigating ligand recognition by the ER-LBD in the event that our NMR studies are not tractable.

Based on our preliminary NMR data collected for assignment, we determined that data should be collected at as high of a field strength as possible. Significant improvement in sensitivity, signal-to-

noise, and the TROSY enhancement are all anticipated when moving from 600 to 800 MHz. Towards this end we have established collaborations with researchers with access to an 800 MHz spectrometer. We have access to 800 MHz spectrometers in the laboratory of Prof. Mark Rance and independently in the laboratory of Dr. Jonathon Moore. However, more routine access to high field spectroscopy on a regular basis is needed. Thus, we (University of Colorado Department of Chemistry and Biochemistry) have established a consortium with the University of Utah Medical School and the University of Colorado Health Sciences Center to create a shared facility with an 800 MHz spectrometer. The three participating institutions will share the time, operating costs and matching costs of the facility equally. Our proposal for this instrumentation is currently under review at both the NIH/NSF Joint Shared Instrumentation program and, independently, at the Keck Foundation. Access to high field instrumentation will significantly enhance the likelihood of obtaining full backbone resonance assignments on this challenging system.

Based on our concern that it might be difficult to obtain full backbone resonance assignments as planned, we are expanding our efforts towards the understanding of ligand specificity of the ligand binding domain into new areas. In addition to the work described above, CD denaturation studies have been conducted on the estradiol and tamoxifen complexes (please see CD denaturation data in the appendix). These data have allowed us to compare the thermodynamic stabilities of these complexes, and we have determined that the midpoint for denaturation is very similar for these complexes. The midpoint for denaturation is ~5.5 M guanadinium hydrochloride (GdnHCl) for the estradiol-bound complex, and ~6 M GdnHCl. As expected from the known structures of the ER-LBD, the CD signal of the folded protein is entirely helical, transitioning to a typical unfolded CD spectrum as chaotropic concentration is increased. The unfolding transition is not a simple 2-state unfolding, and likely involves partially folded intermediates. This behavior is not unexpected for a dimeric protein of this size.

In order to assess the integrity of our estrogen receptor ligand binding domain (LBD) samples, it is important to have an assay to measure the efficacy of ligand binding. In the past, typical binding assays have used whole cell homogenates (*e.g.*, rat uterine cytosol) as a source of unpurified estrogen receptor. In contrast, our goal is to develop a binding assay that can measure the binding efficacy of the purified LBD and any LBD mutants that are used. This assay should be suitable for all ligands being studied, be functional at the conditions we are using for the structural work, and allow for direct comparison of the binding affinity of mutant proteins. Work is presently on-going in the lab in order to develop such an assay. Several published and unpublished protocols are being tried to determine which one will give the most reliable binding assay. Typical binding assays involve the use of a tritiated ligand such as 3H-estradiol and then competing off the tritiated ligand with cold unlabeled ligand. Then, the complex of bound ligand and estrogen receptor needs to be separated from unbound ligand. Typically, the bound complexes are bound to hydroxyapatite and then the bound ligand is eluted with ethanol. The ethanol elution is counted using liquid scintillation counting and the % bound 3H-estradiol is determined. The amount of 3H-estradiol bound in the absence of any unlabeled ligand (after correction for any nonspecific binding) is taken as the 100% bound quantity and the relative efficacy of ligands at competing off the labeled estradiol is determined. In our case, the ligand binding domain does not appear to be binding to the hydroxyapatite and we are presently searching for other techniques for distinguishing bound from unbound ligand. It appears that the use of controlled pore glass (CPG) beads, as recommended by our collaborators the Greene lab at

the University of Chicago Medical School, may hold promise. If this technique does not give reliable results, alternative techniques will be pursued.

Recently, two exciting advances in the understanding of BRCA1 have been made. First, a region of BRCA1 that interacts with the estrogen receptor has been identified. In fact, this region includes the ligand binding domain, and studies are currently in progress to determine if the ligand binding domain is necessary and sufficient for this potentially very exciting link between BRCA1 and ER. Second, the structure of the complex of BRCA1 and its biological partner has been determined. Since we have optimized the protocol to produce recombinant ER-LBD suitable for high resolution structural studies, we are pursuing a collaboration to study the effect of binding the ER-LBD to BRCA1 by NMR. This effort is very preliminary, but may be extremely important if the ER-LBD region is linked to BRCA1 function.

We have not yet begun work on Technical Objective 4. This objective was essentially to repeat the above studies, which are conducted on the ligand binding domain from ER α on ER β . However, until the conditions for the ER α studies are well established, branching out to studies with ER β are not prudent.

KEY RESEARCH ACCOMPLISHMENTS

- Highly purified, stable LBD-ER has been prepared and conditions for expression with a variety of NMR-active isotope labels have been optimized.
- A new protocol for the preparation of ligand-free ER has been explored and evaluated.
- Thermodynamic analysis of protein/ligand complex stability has been conducted.
- Analysis of the differences between ^{15}N - ^1H HSQC NMR data on estradiol and tamoxifen complexes has been initiated.
- The feasibility of obtaining resonance assignments at our highest available field strength has been assessed. Collaborations to obtain data at 800 MHz, as well as an application for funding to acquire 800 MHz spectrometer, have been initiated.
- A binding assay to determine activity at our experimental conditions in a systematic fashion is currently being developed.

REPORTABLE OUTCOMES

Posters presented: The work described in this progress report has been presented at several meetings as either a talk or poster. These include (1) The University of Colorado Annual Retreat (Winter Park, Colorado) (2) The University of Colorado Biophysics Supergroup Meeting and (3) The EPA-STAR Award Annual Meeting (Washington, DC).

Graduate Student Training: Two graduate students, Dana Warn and Aaron Miller, have been trained on this research project. Dana Warn has been primarily responsible for protein expression and purification protocols, and NMR spectroscopy. Her stipend and tuition has been provided through an EPA-STAR fellowship. During the last year of funding, Dana Warn obtained a Master's Thesis in Chemistry and Biochemistry from the University of Colorado entitled "Environmental Estrogens and Breast Cancer Therapeutics: Development of a Technique to Characterize the Diverse Ligand Binding Characteristics of the Estrogen Receptor," awarded December, 2000. Aaron Miller was a first year graduate student who worked on the development of a preparation protocol for the ligand-free estrogen receptor ligand binding domain.

Undergraduate Training: Andrea Wismann, an undergraduate at the University of Colorado, Boulder, conducted independent research on this project during the summer of 2000. Through this research experience, she learned protein expression and purification strategies, as well as circular dichroism spectropolarimetry.

Professional Research Associate Training: The re-optimization of our protein expression protocol and the development of a ligand binding assay has been performed by Leslie Glustrom, a professional research associate in the laboratory who is currently being trained on this project.

CONCLUSIONS

In the second year of funding, progress has been made towards the stated goals. In addition, with further insights derived from our data and the published literature, we have explored new directions in the project. We have developed a more reliable protein expression protocol, have optimized the preparation of estrogen-bound ER-LBD, and have explored protocols that will allow us to obtain estradiol-free ER-LBD. This protein will be essential for studying weakly bound compounds. In addition, due to the lack of consistent data in the literature, we are developing a ligand-binding assay in our lab to directly compare ligand affinities at the experimental conditions used for structural studies. Pilot experiments have indicated that we will likely not be able to obtain NMR resonance assignments at our highest available field strength. We have both established collaborations and applied for funding to obtain time on an 800 MHz instrument. However, we have demonstrated that the strategy of chemical shift mapping at 600 MHz is still feasible with the addition of TROSY pulse sequences and uniform deuteration of the protein. Thus, once resonance assignments are obtained we will be able to rapidly screen both a variety of ligands and medically relevant mutants of the protein.

REFERENCES

No references are included in this report.

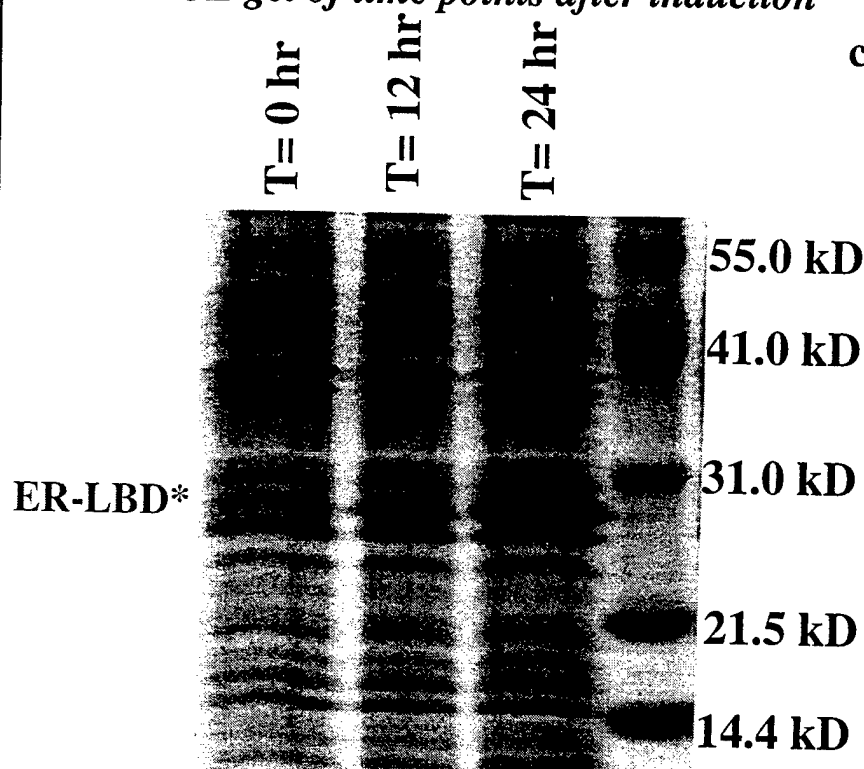
APPENDICES

Figures referred to in the text are appended below.

Production of ER-LBD* for NMR studies

SDS-PAGE gel of time points after induction

E. coli. (BL21(DE3) pLysS)
cells tranformed with plasmid
carrying ER-LBD

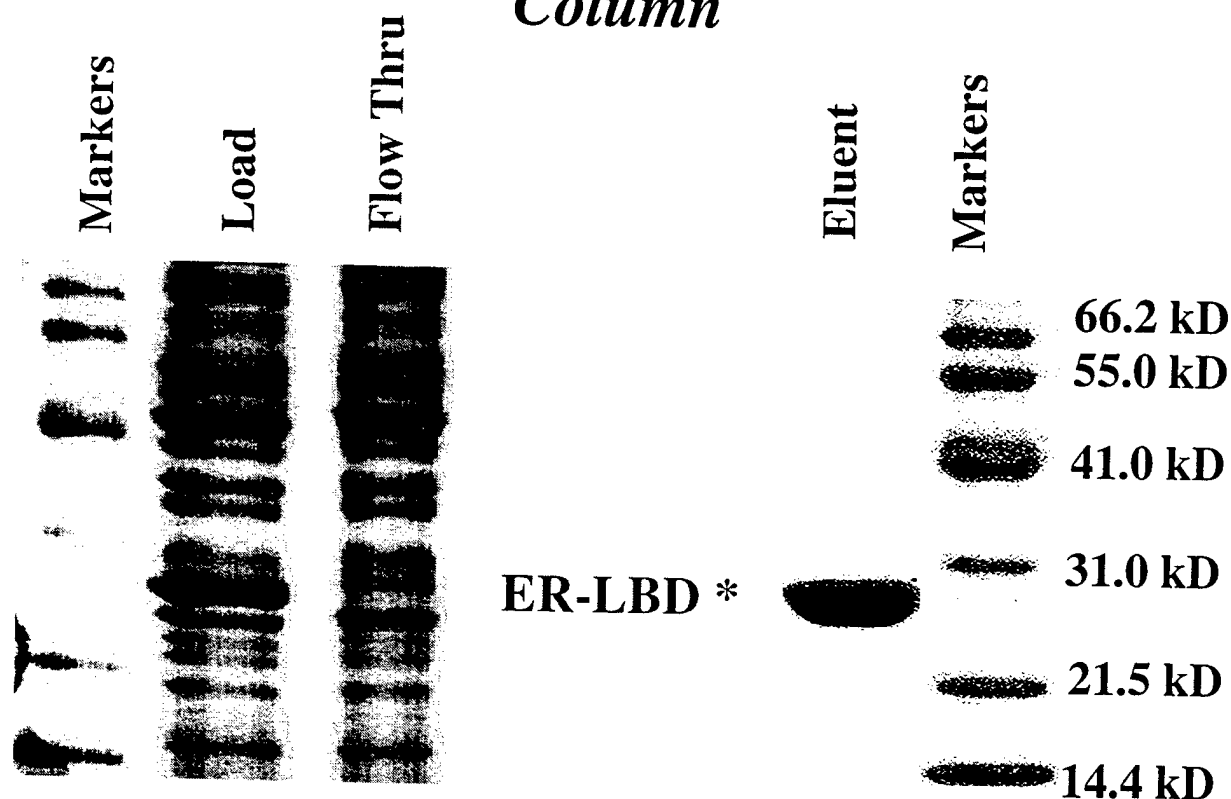


↓
Grow cells in minimal
media in 99% D₂O with
(¹⁵NH₄)₂SO₄ as the sole
nitrogen source, ¹³C
glucose as the sole carbon
source

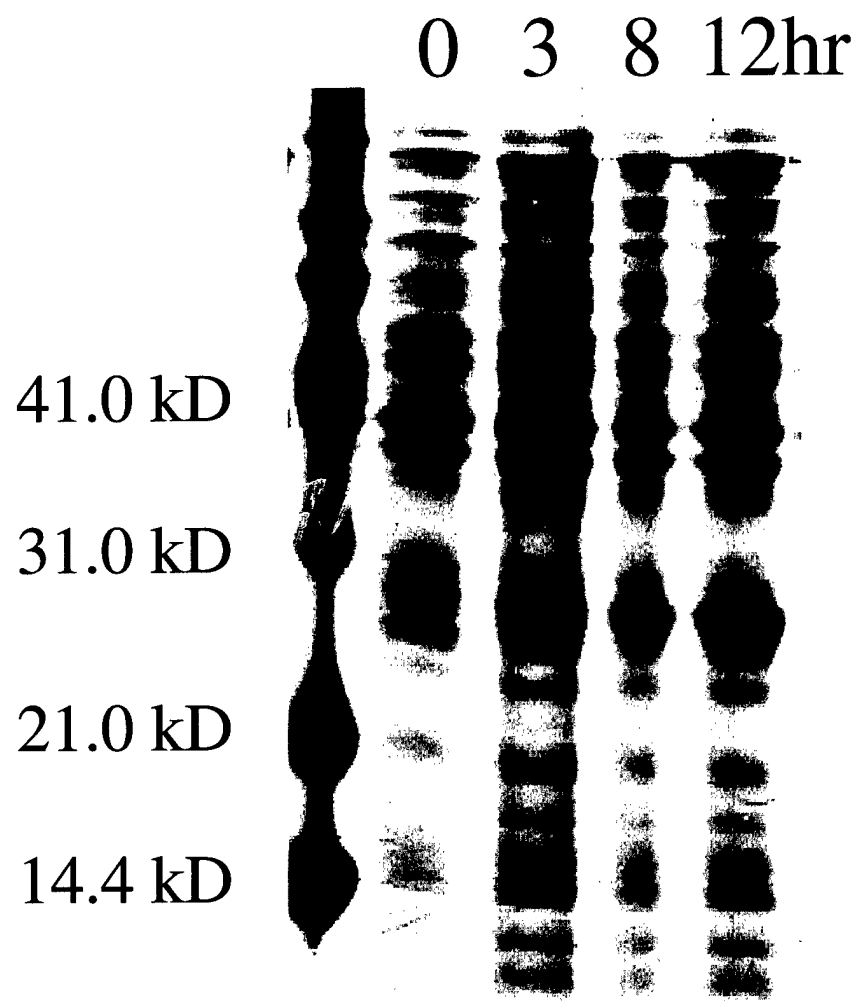
↓
Induce with IPTG

↓
Express labeled ER-LBD

Simplified Purification using the Estradiol Affinity Column



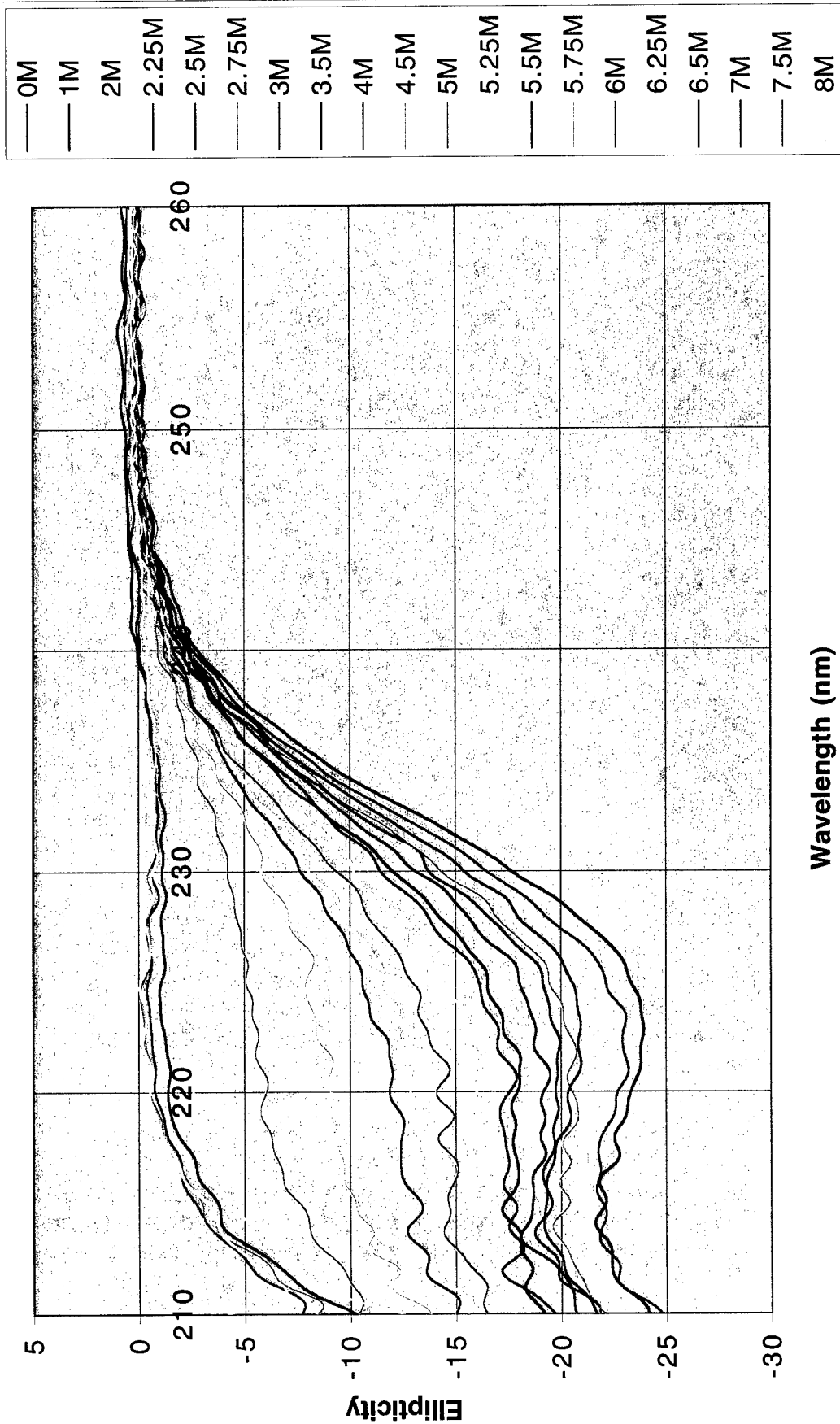
Sample Induction of Partially Deuterated ER-LBD: SDS-PAGE of time points after induction with IPTG



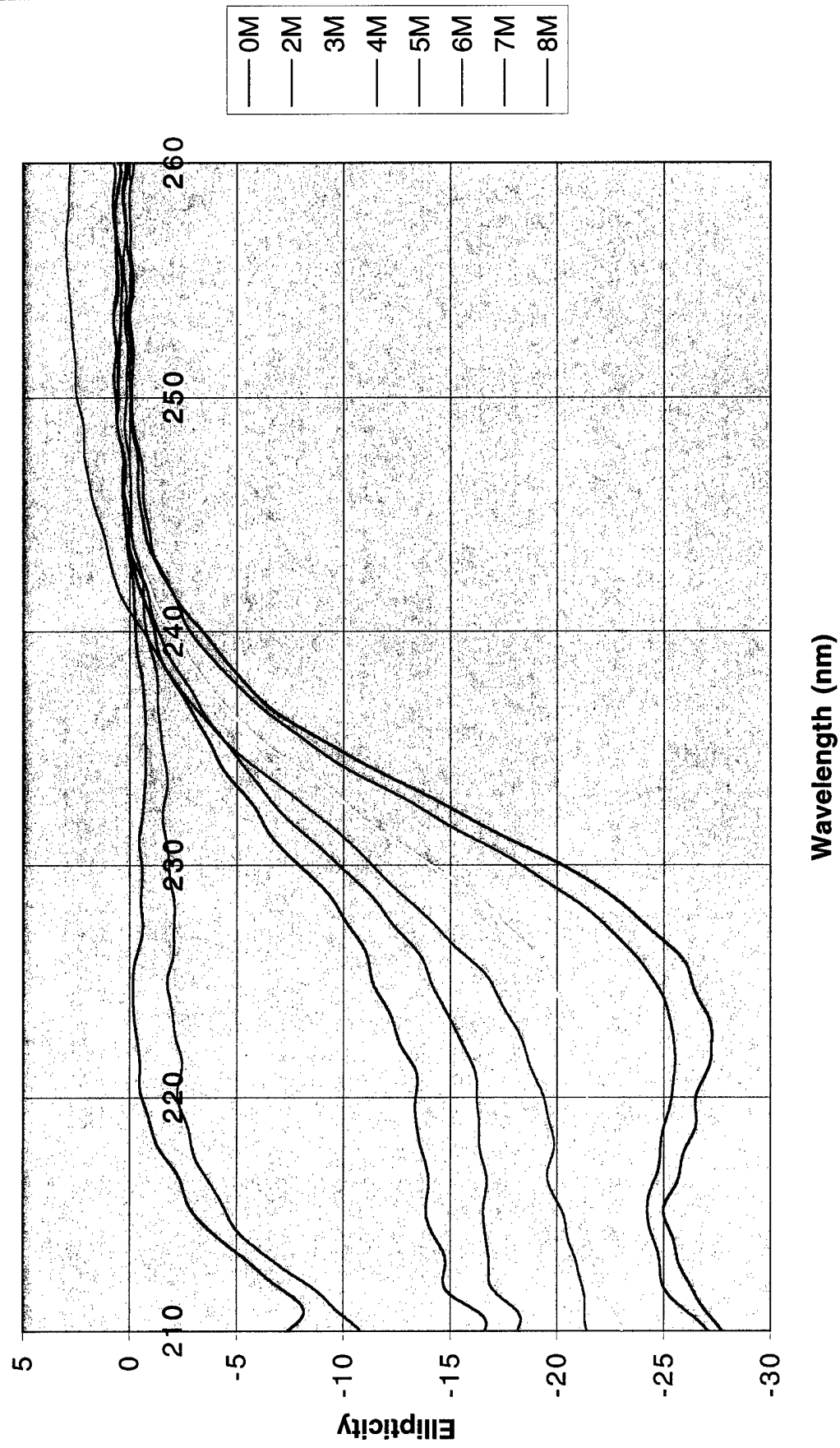
ER Denaturation in GdHCl

The graph displays the circular dichroism (CD) spectra of ER in GdHCl at concentrations ranging from 0M to 8M. The y-axis represents Ellipticity, ranging from -30 to 5. The x-axis represents Wavelength (nm), ranging from 210 to 260. The curves show a characteristic negative peak around 222 nm and a positive peak around 208 nm. As the concentration increases, the magnitude of the negative peak increases, while the positive peak decreases, indicating denaturation of the protein.

Concentration (M)
0M
1M
2M
2.25M
2.5M
2.75M
3M
3.5M
4M
4.5M
5M
5.25M
5.5M
5.75M
6M
6.25M
6.5M
7M
7.5M
8M



ER w/ Tamoxifen Denaturation in GdnHCl

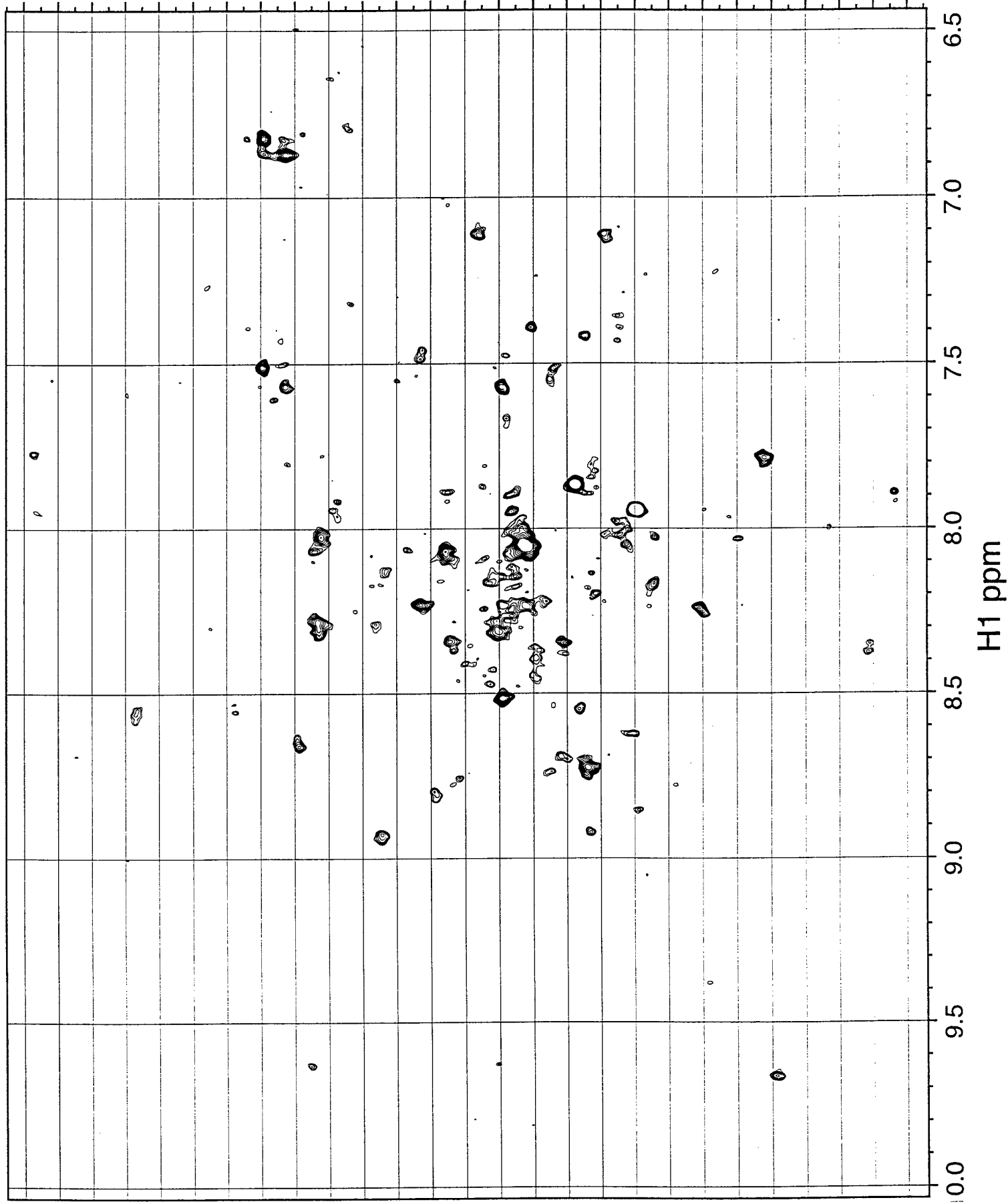


$^1\text{H} - ^{15}\text{N}$ HSQC of ^{15}N ER-LBD Bound to Estradiol
in 20 mM d-Tris, 200 mM NaCl, 1mM d-DTT, 0.02% NaN_3 ,
Taken at 30° C

The HSQC spectrum of the ^{15}N labeled protein shows the broad linewidths expected for a protein of this size. The HSQC-TROSY technique shown in the following spectrum exhibits reduced linewidths and improved spectral quality.

N 1 5 p p m

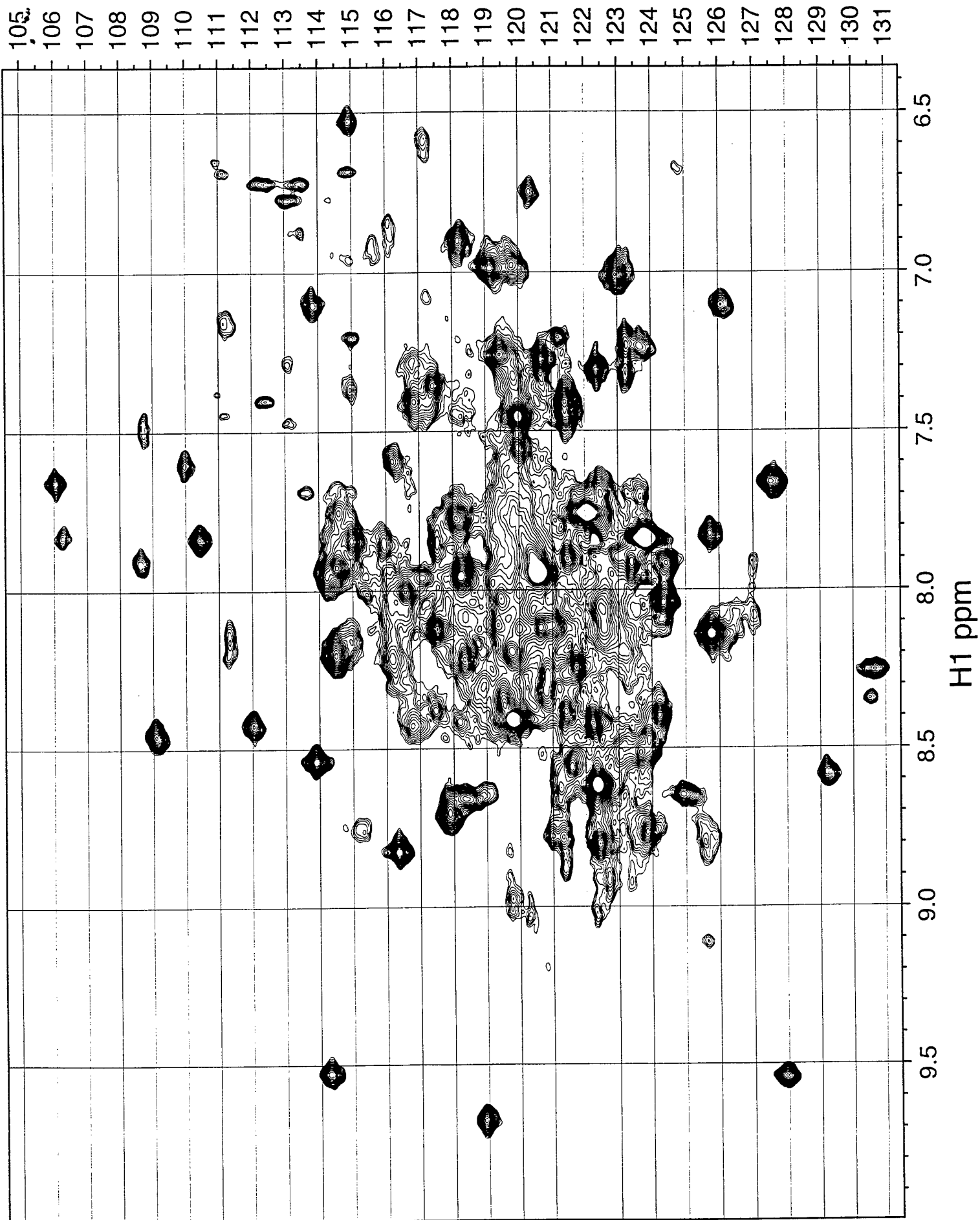
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131



$^1\text{H} - ^{15}\text{N}$ TROSY-HSQC of ^{15}N ER-LBD Bound to Estradiol
in 20 mM d-Tris, 200 mM NaCl, 1mM d-DTT, 0.02% NaN_3
Taken at 30° C

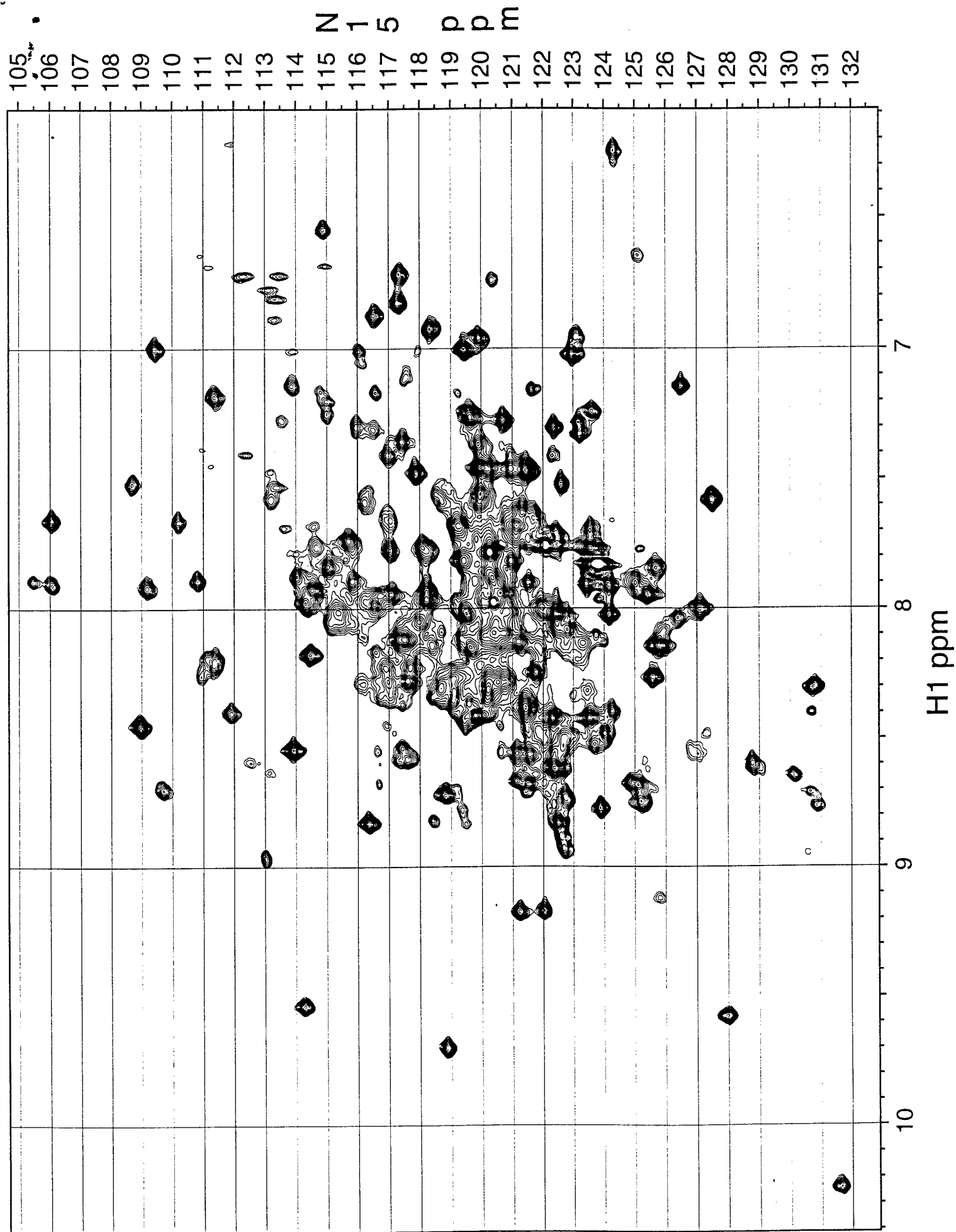
The HSQC-TROSY spectrum produces a significant improvement in the data acquired, producing narrower linewidths and providing more information than the HSQC experiment. In the HSQC spectrum, the ^1H linewidth was 30 Hz; in the HSQC-TROSY spectrum, the ^1H linewidth was reduced to 20 Hz. Nonetheless, the TROSY technique alone at 600 MHz does not provide sufficient narrowing of the linewidth for complete resonance assignment.

N 1 5 p p m



$^1\text{H} - ^{15}\text{N}$ TROSY-HSQC of Partially Deuterated ^{15}N ER-LBD
Bound to 4-OH-Tamoxifen in 50mM d-Tris, 100 mM NaCl,
100mM Na_2SO_4 , 1mM d-DTT, 0.02% NaN_3 , Taken at 30° C

Partial deuteration significantly improves the quality of the data, and will allow for chemical shift mapping. However, it would still not be possible to make full resonance assignments with data taken on a 600 MHz spectrometer. Efforts are now underway to gain access to an 800 MHz spectrometer in order to assess the quality of the data collected at higher field strength.



Overlay of ^1H - ^{15}N TROSY-HSQC of the 4-OH-Tamoxifen-ER-LBD complex, 700 μM , with the Estradiol-ER-LBD complex, 400 μM . Both partially deuterated, ^{15}N , in 50mM d-Tris, 100mM NaCl, 100mM Na_2SO_4 , 1mM d-DTT, 0.02% Azide, taken at 30°C

